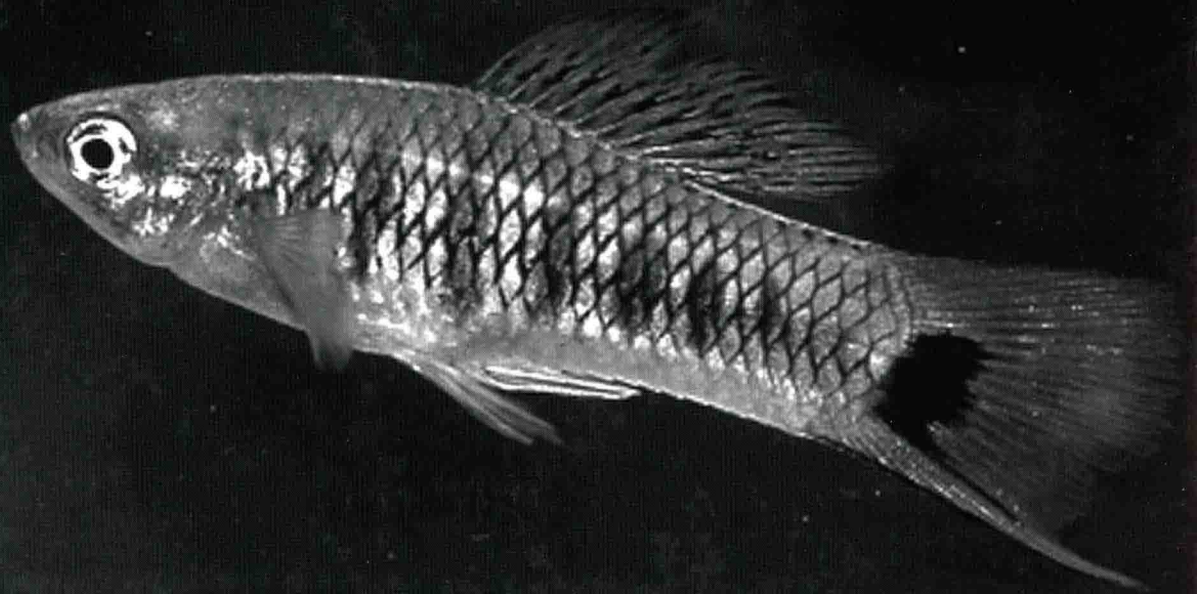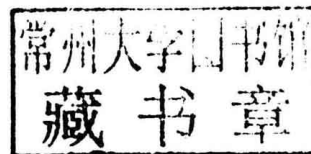# Ecology and Evolution of Poeciliid Fishes

Edited by Jonathan P. Evans,
Andrea Pilastro, and Ingo Schlupp

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Foreword

B Y ALMOST any measure, the Poeciliidae is a fascinating family of fishes of a significance to scientific study greatly out of proportion to their individual sizes (small) or family diversity (medium). Possibly no other family of fishes has attracted so much interest for so many diverse reasons. The scientific areas of investigation that have drawn heavily upon these fishes make up a lengthy list that includes (in no particular order) population and community ecology, life-history evolution, sex determination, viviparity, unisexuality, basic inheritance studies, sexual selection, behavior, social systems, ecological genetics, phenotypic plasticity, parasitology, exotic-species problems, conservation, biogeography, sex linkage, sensory systems, speciation, physiology, immunology, oncology, and . . . the list goes on. Few families have contributed so broadly to scientific investigation of the natural world.

Poeciliids also play a strong role in very practical, species- and ecosystem-level management. Some are the focus of endangered-species recovery efforts, others have gone locally extinct at the hands of humanity, and in other cases they are harmful and destructive exotic invasives, even placing other species of rare poeciliids in harm's way. Some poeciliids are erroneously used in mosquito control and then become major pests; others have been more benignly transplanted outside their native ranges, fortunately with modest effects.

In addition to their scientific importance, poeciliids are of tremendous interest to aquarists, have developed a large and devoted following, and are the focus of lay publications, hobbyist societies, and conferences. They are raised in captivity in huge numbers for the aquarium trade, as well as bred and traded privately among devotees. In some cases, rare species are maintained in captivity by aquarists. Thus, they are an economically important group of fishes. For all these reasons, poeciliids deserve close scientific scrutiny.

It has been 22 years since Franklin Snelson and I edited a volume on these species, *Ecology and Evolution of Livebearing Fishes (Poeciliidae)*, a book now out of print. At that time it had been more than 25 years since Donn Rosen and Reeve Bailey in 1963 published their seminal and classic review of the structure, zoogeography, and systematics of poeciliids, and we felt in 1989 that a collection of chapters on the ecology, reproductive biology, genetics, life-history evolution, systematics, and conservation and impacts of poeciliids was overdue. Consequently, we persuaded some of the top researchers in the world to contribute to that volume, and because of their hard work and knowledge base, the family gained appropriate attention as subjects of scientific study and renewed efforts in the laboratory and the field. Twenty-two years later we are well due for another assessment, and I am delighted to see the volume that follows.

I am fascinated to see how far poeciliid science has come in the intervening 22 years, and this volume is a huge leap forward. The level of sophistication in poeciliid studies has increased tremendously, as has our detailed knowledge of these fishes. The chapters that follow demonstrate in no uncertain terms that this is a fundamentally important group of fishes for understanding how nature operates on levels from molecules to ecosystems. These species are treasure troves of scientific inquiry and continue to enlighten us at

so many levels. I am personally gratified that so much attention is still being paid to this group of species.

When all is said and done, I think that, cold scientific assessments aside, these are simply fascinating fishes that feed our curiosities! Any organism that can have several (sometimes many) broods of offspring developing simultaneously and fed by the mother is well worth our attention. To understand viviparity, superfetation, measurable responses to selection in just a few generations, species interactions, unisexuality, complex color and inheritance patterns, and the dozens of other topics represented in this book is intellectually satisfying and personally exciting. I believe these fishes will continue to capture our attention and imagination for decades to come, and there is little danger of exhausting the deep supply of newly revealed secrets any time soon.

We still have much to learn from this endlessly fascinating family, and this book is a major step in that direction.

The real danger, of course, is in losing populations and species of poeciliids in the catastrophic global declines of nature that we are experiencing; addressing that tragedy is our most urgent calling. We cannot continue to harvest scientific wealth from species that no longer exist, and consequently we must deal immediately with their conservation in situ. If we fail in that effort, then books like this one and the previous volumes simply will become testimonials to our scientific brilliance and our simultaneous failures as caretakers.

Gary K. Meffe

Preface

FEW VERTEBRATE GROUPS have made such significant inroads into multiple fields of research as poeciliid fishes. The value of this family as evolutionary and ecological models is unprecedented and unrivaled among vertebrates and indeed surpasses that of many invertebrate systems as models in genetics, ecology, life-history evolution, and sexual selection. The first synthesis of the emerging body of literature on evolutionary ecology in poeciliid fishes resulted in the publication of Gary Meffe and Franklin Snelson Jr.'s (1989b) edited volume *Ecology and Evolution of Livebearing Fishes (Poeciliidae)*. Our motivation to compile a successor to this volume was simple: for over two decades, poeciliid biologists have relied heavily on Meffe and Snelson's volume, but sadly this has long been out of print, and in the many years since its publication there has been a tremendous amount of new work, encompassing both existing and new topics. Our motivation here is to provide a single reference source that documents these exciting developments. Throughout we have sought reviews that critically evaluate the literature and outline gaps in our knowledge. To this end we have brought together 53 researchers working in North America, continental Europe, Great Britain, Mexico, and Australia, covering subjects that broadly fall into five main research areas, which reflect the subdivisions of this book: (I) reproductive biology and life history, (II) evolutionary ecology, (III) behavior and cognition, (IV) sexual selection, and (V) genetics.

Unlike its predecessor, our volume does not include a chapter on phylogeny and taxonomy. This area has always been in flux and has seen rapid change over the past years, leading to several detailed phylogenetic hypotheses within smaller taxonomic groups. A very broadly based phylogenetic hypothesis addressing the question of the outgroup of all poeciliids and the general zoogeography was proposed recently by Hrbek et al. (2007). This is one of the latest phylogenetic hypotheses that builds on classical work by Rosen and Bailey (1963), Parenti (1989), and many more and is the one most frequently referred to throughout the present volume. The latest hypotheses, however, are more complex than those outlined by the older literature and many questions remain open. Especially important will be attempts to reconcile biogeography and phylogenetics. The current data suggest multiple waves of colonization of Middle America, but not enough detail is known. Another topic that needs additional attention is the phylogenetic position of *Tomeurus gracilis*, the only poeciliid that is not livebearing. This species was thought to be ancestral, but this view has been challenged recently by Hrbek et al. (2007), who suggest that *T. gracilis* has secondarily lost this trait. This has important implications for studies using character mapping. Clearly, more detailed studies are needed not only to understand the bigger picture but also to unravel relationships within and among genera. The list of named species within the poeciliids is still growing, as new species are being described. Clearly, widely distributed species such as *Poecilia mexicana* are likely candidates for finding cryptic species. Describing the diversity within the poeciliids is unfortunately a race against the extinction of species, just as documenting the biogeography of species is increasingly hampered by artificial introductions of species into foreign habitats.

The present volume begins with a section devoted to

reproductive biology and life-history evolution, commencing with an overview of poeciliid reproductive biology (**chapter 1**) by Hartmut Greven. This chapter provides a comprehensive overview of the structural adaptations employed during reproduction and embryonic development, including those involved in maternal-offspring interactions. Such maternal-fetal interactions are explored in detail in **chapter 2**, by Edie Marsh-Matthews, who summarizes recent developments in our understanding of the role of maternal contributions to offspring. Continuing these themes, in **chapter 3** Marcelo Pires and colleagues focus on the adaptive basis and evolutionary origins of such traits, shedding light on what drives their evolutionary diversification across the family. In **chapter 4** Jerald Johnson and Justin Bagley employ a comparative framework to infer links between life-history evolution and agents of natural selection, primarily focusing on studies that document intraspecific life-history diversification. Part I concludes (**chapter 5**) with Ingo Schlupp and Rüdiger Riesch's review of one of the more unusual aspects of poeciliid biology: unisexual reproduction, a mode of reproduction that was thought to be impossible in vertebrates until Laura and Carl Hubbs described sperm-dependent, clonal reproduction in *Poecilia formosa* in 1932.

Part II, on evolutionary ecology, commences with Gregory Grether and Gita Kolluru's review (**chapter 6**) examining the role of resource availability as an agent of natural selection, arguing that such effects, although largely overlooked in the literature, may have profound evolutionary implications. In **chapter 7**, Seth Coleman reviews a growing body of literature that investigates the use of specific sensory modalities in poeciliid behavior and the genetics and physiology of poeciliid sensory receptors, in particular of the visual system. Joanne Cable (**chapter 8**) then reviews the literature on poeciliid parasites—a rich and fascinating area—and explores the evolutionary interactions of hosts and parasites and highlights the importance of parasites in both wild and captive poeciliids. In **chapter 9** Joel Trexler and colleagues develop a new theory that integrates community assembly and parental care in order to predict the distribution of livebearing fishes. Next, Gil Rosenthal and Francisco García de León (**chapter 10**) explore the mechanistic and ecological factors that influence reproductive isolation, speciation, and hybridization. In **chapter 11** Michael Tobler and Martin Plath review the ways in which poeciliids have adapted to extreme environments such as hydrogen sulfide–rich and cave habitats. Part II concludes with Craig Stockwell and Sujan Henkanaththegedara's review of the emerging field of evolutionary conservation biology, an approach geared toward conserving species in the context of ecological and evolutionary processes (**chapter 12**).

Part III focuses on behavior and cognition and commences with a chapter by Jens Krause and colleagues (**chapter 13**), who consider the factors driving shoal composition and grouping behavior and explore recent methodological advances for quantifying such interactions. The next two chapters recognize the increasing use of poeciliid fishes as models for understanding learning and cognition. In **chapter 14** Mike Webster and Kevin Laland review the myriad ways in which poeciliid fishes learn and innovate in order to extract and exploit information from conspecifics or their environment. Next, in **chapter 15** Angelo Bisazza considers the complex cognitive abilities of poeciliids, including memory, spatial organization, numerical abilities, and lateralization of cognitive function. To conclude part III, Jennifer Kelley and Culum Brown look at predation risk and decision making in poeciliid prey, evaluating the evidence that predation risk is an important factor driving the evolution of prey cognition (**chapter 16**).

Part IV turns to sexual selection, starting with two chapters that review the two broad mechanisms of this evolutionary process: intra- and intersexual selection. In **chapter 17** Oscar Rios-Cardenas and Molly Morris explore how both mechanisms of sexual selection operate *before* mating, through mate choice and intrasexual competition, and how they interact to influence male mating success. In **chapter 18** Jonathan Evans and Andrea Pilastro focus on both mechanisms as agents of sexual selection *after* mating, in the form of sperm competition and cryptic female choice, and examine how both processes influence patterns of male reproductive success. The next chapter (**chapter 19**), by Anne Magurran, focuses on sexual coercion—a prominent feature of poeciliid mating systems—and explores its costs for females, the ecological settings in which it occurs, and the reasons for individual differences in the responses to coercion. In **chapter 20**, in their review of communication networks and sexual selection, Matt Druen and Lee Dugatkin explore how socially acquired information (eavesdropping) expands our understanding of mate choice and aggression. Part IV concludes with a chapter on genital evolution by Brian Langerhans (**chapter 21**), who evaluates several hypotheses proposed to account for the extraordinary diversity of male genital form in poeciliid fishes.

Part V combines papers on a broad array of subjects involving genetics. One of the extraordinary features of poeciliids is the high degree of phenotypic variation among populations and species. Since the publication of Meffe and Snelson's book, DNA markers have almost completely supplanted allozyme markers. In their opening chapter (**chapter 22**), Felix Breden and Anna Lindholm survey what we know in the DNA era about genetic structure of natural populations of poeciliids, looking for the signatures that sexual selection, natural selection, migration, and/or drift leave on the population phenotypic and genetic variation.

In **chapter 23** Robert Brooks and Erik Postma focus on guppies as an evolutionary model for understanding the inheritance of color pattern genes, the genetic basis for their expression, and the processes thought to underlie the extreme polymorphism in these traits. In **chapter 24** Manfred Schartl and colleagues provide an overview of sex determination in poeciliids, highlighting the fascinating diversity of sex-determining mechanisms in this group. This is followed by Mark McMullan and Cock van Oosterhout's chapter (**chapter 25**) on the major histocompatibility complex (MHC), a large multigene family involved in immunocompetence. Using data from the guppy and other poeciliid species, they investigate the role of natural selection (parasite resistance), sexual selection, and selection on linked mutations that hitchhike with the MHC alleles in the maintenance of MHC polymorphism. Finally, in **chapter 26** Manfred Schartl and Svenja Meierjohann focus on how poeciliids are used in cancer research. The workhorse of this research is still the classical Gordon-Kosswig melanoma system, but this chapter goes well beyond traditional genetics and highlights recent advances in understanding skin cancer.

The final chapter of the book, **chapter 27**, by John Endler, is an integrative commentary that critically evaluates the state of the field while highlighting fruitful directions for future research. We are delighted that John has agreed to provide such a commentary, and we can think of no one more suitable to stimulate the next generation of evolutionary and ecological biologists working on this fascinating family of fishes. If our volume serves its purpose, and as John himself hints in the final chapter, we will no doubt see a third volume on the subject in years to come.

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Acknowledgments

Contents

Part I

Reproductive biology
and life history

Chapter 1 Gonads, genitals, and reproductive biology

Hartmut Greven

1.1 Introduction

ALL POECILIIDAE, or Poeciliinae (Parenti 1981; Hrbek et al. 2007), give birth to competent fry, and their mode of reproduction is traditionally named viviparity. Only one rarely studied species, *Tomeurus gracilis*, appears to be more plastic in this respect, primarily laying eggs with embryos at an early stage of development (e.g., Rosen & Bailey 1963). The evolution, adaptive value, and benefits of viviparity and associated costs, most of which accrue to the gravid mother, have been reviewed repeatedly elsewhere (Pires et al., **chapter 3**; Marsh-Matthews, **chapter 2**) and are therefore not considered in this chapter.

Structural adaptations to viviparity are diverse, but their treatment in the recent literature is largely limited to a few common species. Other than the copulatory organ, which is frequently used for species classification, the structures involved in reproduction are poorly described in many poeciliids. However, some generalizations are possible, and in this chapter I briefly summarize this information. I focus on primary sex characteristics, that is, the gonads, and some secondary sex characteristics, such as genital ducts and external genitals. I also point out structural adaptations involved in maternal-fetal exchange, touch upon parturition and embryonic development, and point out gaps in our knowledge.

1.2 Development

1.2.1 Gonads and gonadal ducts

Determination of gonadal sex (i.e., primary sex determination) in gonochoristic poeciliids, studied in a few species only, involves the formation of either an ovary or a testis and development of intragonadal ducts from the primarily bipotential gonad (e.g., *Xiphophorus* [formerly *Platypoecilus*] *maculatus*: Wolf 1931; Schreibman et al. 1982; *Poecilia reticulata* [formerly *Lebistes reticulatus*] [guppy]: Weishaupt 1925; Goodrich et al. 1934; Dildine 1936; Anteunis 1959; *Xiphophorus hellerii*: Essenberg 1923; *Gambusia affinis*: Koya et al. 2003). Primordial germ cells (PGCs) are specified by determinants found in the germinal plasma that can be identified in very early developmental stages. During embryonic development PGCs migrate from their extragonadal position in the genital ridge on each side of the dorsal mesentery to form a pair of gonadal primordia. These primordia consist of somatic cells, which are derived from the peritoneal wall, and germ cells, which are derived from PGCs. Proliferation of germ cells gives rise to a stem-cell population of oogonia or spermatogonia.

The first sign of prenatal sex differentiation of the still paired primordia is the larger size or larger number of germ cells distributed in the prospective ovary or the larger number of somatic cells, especially in the hilar region, of the prospective testis, with germ cells located at the periphery. Differentiation into ovarian or testicular tissue can take place before birth (Goodrich et al. 1934; Dildine 1936; *G. affinis*: Nakamura et al. 1998) or after birth (e.g., Schreibman et al. 1982), although this may depend on the species.

Before fusion, somatic cells form an intragonadal duct in each primordium. After fusion, intragonadal ducts of the female unite to form a single ovarian cavity (fig. 1.1), which continues in a short extragonadal gonoduct. In sexually mature females the gonoduct ends blindly in the connective tissue between the urethra and hindgut and opens
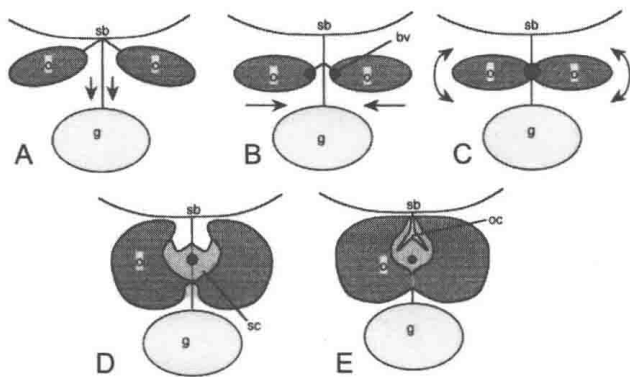
Figure 1.1 Development of the ovary in the western mosquitofish. (A) The paired gonadal primordia hang from the dorsal coelomic wall. (B) Primordia have approached just after birth. (C) Separate primordia fuse at the hilar region. (D) The lateral side of the dorsal stroma elongates upward to the coelomic wall, and the outer part of the ovary bends dorsally. (E) Elongated lateral sides of dorsal somatic cells fuse at the top of the ovary to form the ovarian cavity. bv = blood vessel; g = gut; o = gonadal primordium; oc = ovarian cavity; sb = swim bladder; sc = somatic cell cluster. From Koya et al. 2003. (Figure reproduced with permission of the Council of the Academic Societies, Japan, and Dr. Y. Koya.)

into the urogenital sinus, where the urethra also drains (see section 1.4.3).

In the male the two primordia fuse incompletely, forming a bilobed testis. Branches (efferent ducts) of the paired intragonadal ducts (testicular ducts) extend toward the periphery, where nests of spermatogonia accumulate. Posteriorly the testicular ducts merge to form a common, short, extragonadal vas deferens, which enters the urogenital sinus. The epithelium lining the ovarian cavity and that forming the spermatocysts (see section 1.3.1) are referred to as germinal epithelia, consisting of somatic cells and of oogonia or spermatogonia, respectively.

Extragonadal ducts arise from an anterior (the posterior parts of the gonadal primordium consist of somatic cells only) and a posterior primordium of peritoneal origin, which join together with the gonad later (see also Anteunis 1959). Although not studied in detail, the mature gonoduct appears to consist of two structurally different portions, an anterior aglandular and a posterior secretory portion (M. Uribe, pers. comm.).

Development of the extragonadal ducts is likely to be under the control of gonadal hormones. As shown histochemically, steroidogenesis already occurs in the gonads of newborns (*Poecilia* [formerly *Mollienesia*] *latipinna* [black molly]: Hurk 1974; *X. maculatus*: Schreibman et al. 1982).

In some species gonads contain oocytes surrounded by follicle cells before birth. During their subsequent reversal, the testes appear to be hermaphroditic. Temporary feminization (juvenile hermaphroditism) is not consistently observed among poeciliids (Goodrich et al. 1934; Dildine 1936), and the presence or absence of this phenomenon

may differ among populations, for example, in *G. affinis* (Koya et al. 2003).

Generally, sex differentiation is controlled genetically (for a review, see Devlin & Nagahama 2002; Schartl et al., **chapter 24**), but in some species it is influenced by environmental factors during early stages of gonadal differentiation, for example, pH variation in *X. hellerii* (Rubin 1985). Furthermore, higher temperatures usually result in the production of more males, while lower temperatures result in relatively more females (*Poeciliopsis lucida*: Sullivan & Schultz 1986; Schultz 1993; *Poecilia* [formerly *Limia*] *melanogaster*: Römer & Beisenherz 1996; *Cnesterodon decemmaculatus*: Johnen 2006). Thus, by actively selecting a certain temperature range, females may control the predominant sex of their offspring (Greven, unpublished data). It is likely that temperature-dependent sex determination is more widespread in poeciliids than currently documented. The stage of gonadal differentiation at which environmental sex determination occurs and the length of time the female has to be exposed to the sex-determining factors have yet to be determined.

The time to sexual maturity varies greatly among poeciliids. Maturation and size may be controlled genetically (e.g., *Xiphophorus* spp.; summarized in Kallman 2005), but these factors can also be influenced by the social environment (Snelson 1989). Males are sexually active before the gonopodium is completely developed and before the production of spermatozeugmata (unencapsulated sperm bundles) has begun (e.g., the mosquitofish *G. affinis*: Bisazza et al. 1996; the guppy *P. reticulata*: Evans et al. 2002b).

1.2.2 External genitals

External genitals are secondary sex characteristics. The development of the sexually dimorphic anal fin, which transforms into a copulatory organ (gonopodium) in the male, and of its (internal) axial and appendicular skeletal support (suspensorium) has been studied in species with bilaterally symmetrical "armed" gonopodia (see section 1.3.4 below and, e.g., Essenberg 1923; Turner 1941a/1941b, 1942; Hopper 1949; Rosa-Molinar 2005). At birth, both structures (anal fin and suspensorium) are identical in males and females. Unfortunately, studies on the development of structural adaptations of external genitals in females are not available.

In the guppy, transformation of the anal fin into a gonopodium takes place after differentiation of the testis, and developmental stages of the gonopodium correlate with the degree of maturation and steroid histochemistry of the testis (e.g., Schreibman et al. 1982). This transformation primarily affects anal-fin rays 3, 4, and 5 and occurs according to the following specific temporal sequence, as
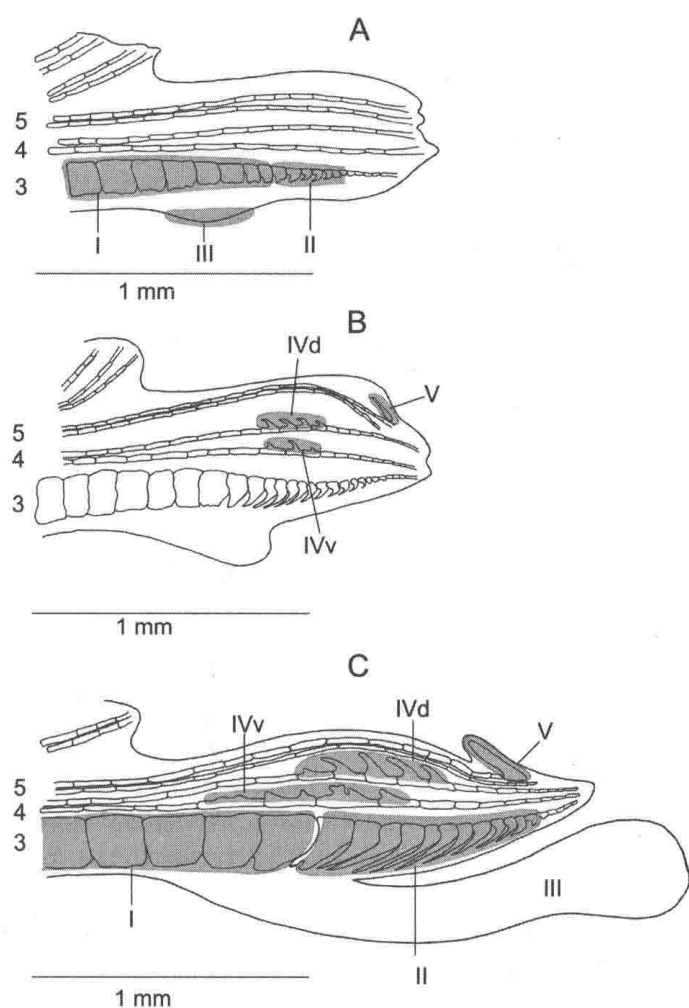
Figure 1.2 Development of the gonopodium of *Poecilia reticulata*. (A, B) The transforming anal fin and (C) mature gonopodium; 3, 4, 5 = anal-fin rays. Accessory structures (shaded) are numbered in the order in which they appear during development: I = thickening of ray 3; II = formation of spines ventrally to ray 3; III = formation of the hood; IVd = spines on the dorsal branch of ray 4; IVv = spines on the ventral branch of ray 4; V = formation of the terminal hook on ray 5. Redrawn from Hopper 1949.

depicted in fig. 1.2: (I) thickening of ray 3; (II) formation of spines ventrally to ray 3, and (III) formation of the hood at the level of segment 1–15 of ray 3; (IV) formation of three areas consisting of a series of spines on the dorsal border of the dorsal branch of ray 4 (IVd) and a series of spines along the dorsal border of the ventral branch of ray 4 (IVv); and (V) formation of a terminal hook at the distal end of the dorsal branch of ray 5. At rest the gonopodium points caudally. In bilaterally symmetrical gonopodia, ray 3 lies below rays 4 and 5 (fig. 1.2; Hopper 1949).

Development of the suspensorium involves the gradual dissolution of the anterior hemal spine(s) and elongation of the interhemal spines (gonactinosts) that articulate ventrally with the gonopodial rays through baseosts. Baseosts carry the muscles that move the fin and are anchored to hemal spines 2, 3 and 4 (gonapophyses I, II, III). The gonapophyses enlarge to form posterior outgrowths (uncini), to which

ligaments attach. A tough network of ligaments holds the gonapophyses, gonactinosts, and baseosts together firmly (see Rosen & Bailey 1963).

A region of six vertebrae (11–16) has been identified as the "genital area" in *G. affinis*. Administration of testosterone to late embryos and adult females induces the anterior displacement of this zone. This shift, as well as growth, elongation, and anterior bending of hemal spines of vertebrae 14–16 (gonapophyses) and the resorption of the 13th hemal arch, leads to the permanent anterior translocation of the anal fin and the suspensorium (Rosa-Molinar et al. 1994). This process is probably mediated by external forces exerted by the interosseal and suspensory ligaments (Rosa-Molinar et al. 1998) and is accompanied by the formation of a sexually dimorphic ano-urogenital nerve plexus of the anal-fin musculature (Rosa-Molinar 2005).

Studies on the development of the enlarged internal and superficial muscles that enable the circumduction of the gonopodium do not exist.

1.3 Male reproductive system

The reproductive system of adult males includes the testis; intragonadal ducts (efferent ducts and paired testicular ducts, or main sperm ducts); the single extragonadal duct (vas deferens) running to the genital papilla (fig. 1.3), which opens into the urogenital sinus; the urogenital aperture, a transversal, or horseshoe-shaped, slit at the origin of the gonopodium; and the suspensorium.

1.3.1 Organization of the mature testis

The relatively large testicular lobes are covered by a thin connective tissue capsule (tunica albuginea), which encloses (1) interstitial tissue, that is, fibroblasts, nerve fibers, smooth-muscle cells, blood vessels, cells of the immune system, and steroid-secreting Leydig cells (reviewed by Grier et al. 1981; Grier et al. 2005), which are dispersed mainly in the space between the branches of the efferent ducts (Pandey 1969; Fraile et al. 1992); (2) testicular lobules (tubules, according to some authors) that terminate blindly at the periphery of the testis and are bordered by an incomplete layer of "myoid" boundary cells (Grier et al. 1981; Arenas et al. 1995b); and (3) the duct system. Branching efferent ducts, which meet testicular lobules, radiate from the main sperm ducts in the center of each lobe (figs. 1.3 and 1.4A and B).

Spermatogonia are restricted to the blind ends of lobules (restricted type of testis). The germinal epithelium forms spermatocysts within the lobules and does not border on a lumen ("epitheliod" instead of "epithelial" testis: Grier et al. 1981; Grier et al. 2005). The restricted testis type and
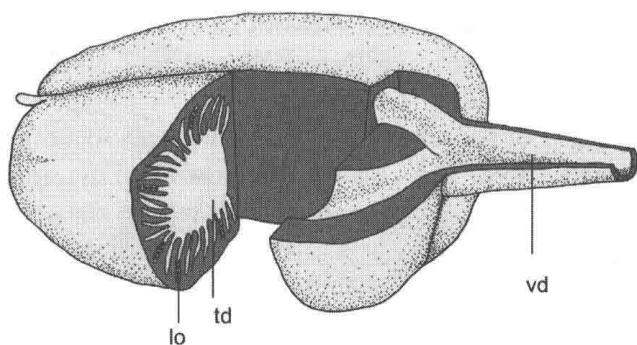
Figure 1.3 Organization of the testis in *Poecilia reticulata*. Lobules (lo) with cysts (black circles); the proximal efferent ducts open into the testicular duct (td). Testicular ducts open into the vas deferens (vd). Redrawn from Billard 1986.
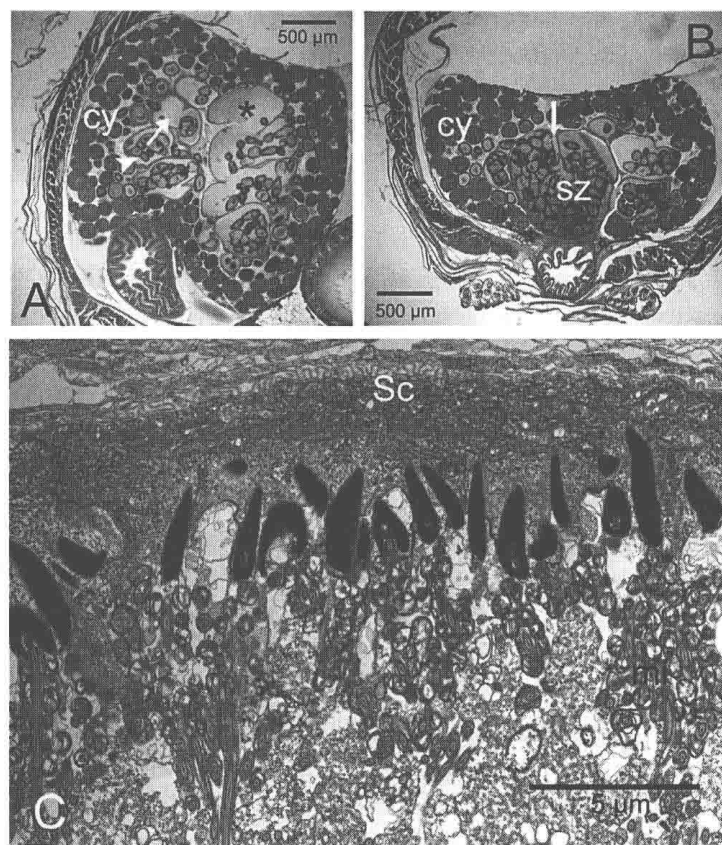

Figure 1.4 Testis and spermatids in *Poecilia* spp. Histological cross sections of (A) the anterior portion of the mature testis of *P. reticulata* with testicular duct (asterisk) and (B) posterior portion with incompletely (arrow) merged ducts; efferent ducts (arrowheads), spermatozeugmata (sz), cysts (cy) with developing sperm. Transmission electron micrograph of late spermatids (C) of *P. melanogaster* with their electron-dense heads embedded in Sertoli cells (Sc).

the epitheliod germinal epithelium are apomorphies of the Atherinomorpha (Parenti & Grier 2004).

New cysts are formed at the distal end of a lobule and contain the synchronously developing stages of spermatogenesis (spermatogonia, spermatocytes, spermatids) and spermiohistogenesis, that is, the final formation of mature spermatozoa from spermatids, during which the elongating

nuclei become tightly associated with Sertoli cells, the somatic portion of the germinal epithelium (fig. 1.4C). During maturation, cysts progress from the distal end down the tubules toward the center of each testis lobe, while the number and size of Sertoli cells increase markedly (Pandey 1969; Grier et al. 1981). Development from meiosis to mature spermatozeugmata (see section 1.3.2) requires at least 35 days (black molly: DeFelice & Rasch 1969).

By the time spermiohistogenesis is complete, cysts are positioned near the efferent-duct system and spermiation begins. During spermiation Sertoli cells transform into secretory efferent-duct cells (Pandey 1969; Grier et al. 2005). The secretions are complex glycoconjugates (Grier et al. 1981; Greven 2005), which hold immotile sperm together to form spermatozeugmata. Each cyst produces a single spermatozeugma, in which sperm heads are external and the tails form the center (Philippi 1908) (fig. 1.4A).

Thus, Sertoli cells support cysts, nourish the germ cells, phagocytose residual bodies that are cast off by maturing spermatids, form spermatozeugmata, transfer and eventually convert metabolites or hormones into germ cells, and establish the blood-testis barrier after meiosis (Marcaillou & Szöllosi 1980; Grier et al. 1981; Bergmann et al. 1984; Billard 1986). In addition, they transport spermatozeugmata, as evident by an increased positive actin immunoreaction in Sertoli cells during spermiogenesis and a strong reaction in efferent-duct cells (Arenas et al. 1995a).

1.3.2 Spermatozeugmata and spermatozoa

Free spermatozeugmata are present in the efferent ducts and are stored in the remaining duct system (fig. 1.4A and B). Storage time might be limited in sexually active males but is considerably longer during the quiescent period in species living in temperate zones (see section 1.3.3).

Strippable spermatozeugmata can vary in size both among individuals of a given species and among species. In ejaculates stripped artificially from male guppies, individual spermatozeugmata measure between 125 and 235 µm in length (Kuckuck & Greven 1997). The number of strippable spermatozeugmata also exhibits considerable variation, ranging from 20 in *Heterandria formosa* to 3000 in *X. hellerii*. Similarly, there is considerable variation in the number of spermatozoa per spermatozeugma among species, ranging from 4000–5500 in *X. hellerii* to up to 34,800 in guppies (Kuckuck & Greven 1997; Greven 2005). Although there can be considerable intraspecific variation in the number of spermatozoa per spermatozeugma (e.g., guppies: 9600–34,800; Kuckuck & Greven 1997), such variation is not systematically explained by male identity (Evans et al. 2003b).